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Title:

Real-time electrochemical detection of paracetamol interaction with intestinal tissue

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Oral drug delivery is the preferred route for drug administration mainly due to good patience compliance. A myriad of approaches is already in use to study the effect of drugs in biological systems, however, there is a constant need for new methods and tools to study their interaction in various parts of the gastrointestinal tract[1,2]. Several *ex-vivo* approaches are used for the evaluation of drug permeation through the small intestine. However, very little is known about the effect and interaction of drugs between the enzymes (e.g., native catalase) present in the intestine. In the current study, we show the application of an electrochemical O_2 sensor for studying the effect of paracetamol on the native catalase in the intestinal tissues.

The sensors, prepared by mounting the cleaned tissue isolated from porcine on a custom-made Clark type electrode, (Fig. 1a) were exposed to different concentrations of H_2O_2, the substrate for catalase. Addition of H_2O_2 can also mimic local changes in redox environment in the tissue, like in the case of inflammation, which results in increased H_2O_2 levels[3].

The observed current change, production of O_2, is due to the reaction of H_2O_2 with catalase (Fig. 1b). Our experiments indicate that the intestinal tissue contains a significant amount of catalase, considering the current generated after the addition of 500 µM substrate. We observe that there is a linear relationship between H_2O_2 concentration and current response (Fig. 1c). Additionally, we demonstrated the antioxidant capacity of paracetamol, which can be seen from decreasing O_2 production (Fig. 1d).

To the best of our knowledge, we present for the first time a method for direct electrochemical measurement of drug-catalase interaction in intestine. As a next step, we intend to further study the effects of other antioxidants and pharmaceuticals on catalase activity in intact tissues.

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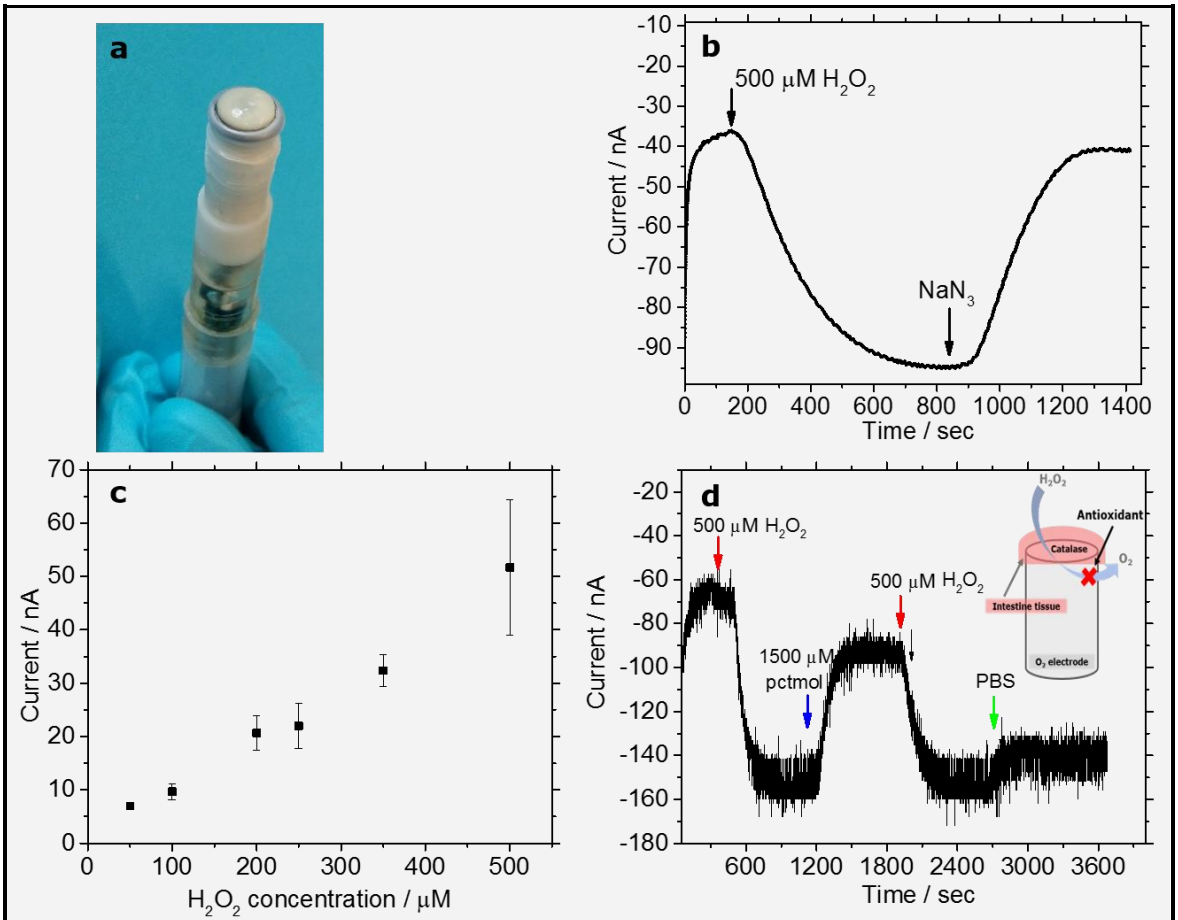

Fig. 1 a) Photograph of O_2 electrode covered with intestinal tissue. **b)** Current recorded from ~~of~~ tissue-covered electrode in phosphate buffered saline (PBS), pH 7.4. Steady-state baseline current (time interval: 100-200 sec) is due to dissolved O_2 in PBS. At 200 sec, H_2O_2 is added resulting in 500 µM H_2O_2 final concentration. At 900 sec, a known catalase inhibitor NaN_3 is added causing the current to return to the baseline. **c)** Linear dependency of current recorded from tissue-covered electrode after addition of different H_2O_2 concentrations. **d)** Amperometric response of tissue-covered electrode with addition of H_2O_2 at 500 sec.-Subsequent addition of paracetamol into PBS resulting into 1500 µM final concentration, caused increase of the current (due to decreasing O_2). The inset shows the schematic view of O_2 inhibition by antioxidants on tissue-covered electrode.

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